Formation of sunflower oil emulsions stabilized by whey proteins with high-pressure homogenization (up to 350 MPa): effect of pressure on emulsion characteristics

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(Received 9 August 2000; Accepted in revised form 6 February 2001)

Summary A new ultra-high-pressure homogenizer was used to make very fine oil in water emulsions. The effect of pressures up to 350 MPa on sunflower oil (20%) in water emulsions was studied. The emulsifier used was whey protein concentrate (1.5%). The properties of the emulsions were characterized by laser light scattering (droplet size distribution) and coaxial cylinders rheometry (rheological behaviour). The protein adsorption fraction was obtained by a spectrophotometric method using bicinchoninic acid reagent.

Significant modifications in the structure and the texture of the emulsions were observed as the pressure increased. No change was revealed by polyacrylamide gel electrophoresis of the whey protein within the pressure range studied. Microdifferential scanning calorimetry scans indicated that the changes of the structural and textural properties may be because of changes in the protein conformation.

Keywords Droplet size, emulsification, microdifferential scanning calorimetry, protein adsorption, viscosity.

Introduction

A large variety of foods are emulsions, from the more natural, e.g. milk, to the more sophisticated, e.g. sausages, mayonnaises. Emulsions are dispersions of liquid droplets in a liquid continuous phase. As the two liquids are immiscible, emulsions are very unstable. In order to stabilize emulsions, there must be surface active molecules at the interface of the droplet to prevent instantaneous coalescence.

Food emulsions are commonly produced in high-pressure homogenizers, in colloid mills or in batch reactors with high-speed blenders. Initially built for the homogenization of milk, high-pressure homogenizers are the most often used, as they give fine emulsions with precise texture properties (creams, ice creams) and higher degrees of stability. The principle of high-pressure homogenization is simple: a coarse emulsion produced with a high-speed blender is forced under pressure through a narrow valve. The combination of the intense shear, cavitation and turbulent flow conditions in this valve leads to the disruption of fat globules (Walstra & Smulders, 1997; McClement, 1999). The decrease of the average size of the fat globules reduces the creaming velocity (Stokes law) and increases the stability of the emulsion. Food homogenizers usually go up to 60 MPa and the gap of the valves is typically between 15 and 300 μm. Increasing the pressure and decreasing the gap size cause a greater degree of breakdown of droplets. Despite the large use of high-pressure homogenizers, few studies deal with the effect of very high pressures on the emulsions properties. Moreover, the studies are limited in the pressure range. According to Mulder & Walstra (1974) and Phipps (1975), the average fat globule diameter (d) decreases with emulsification pressure (P) in a relation $d\alpha P^{-0.6}$, for pressures between 0.25 and 40.5 MPa. Davies (1985) described the breakdown of fat droplets with the Kolmogoroff theory and found a relation between the maximal droplet size

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and $P^{0.4}$. According to Tornberg (1980), for pressures over 40 MPa and for a mass oil fraction of 12%, a phenomenon called 'overprocessing' occurs: the average droplet size increases with pressure. Robin *et al.* (1992) processed butter oil in water emulsions, with sodium caseinate as emulsifier, from 7.8 to 76.3 MPa in a microfluidizer. In the microfluidizer, two microsteams were projected under pressure against one another. They showed that the average size of fat globules decreased with pressure and reached a minimum around 60 MPa. Until now, no study has related the effect of 'dynamic' high pressures over 100 MPa on food emulsion formation. The effects of isostatic pressure on emulsions has been studied as a means of preservation. It has been shown that its influence on the already formed emulsions depends on the emulsifiers used (Dumay *et al.*, 1996).

Thus, the purpose of this work was to test the hypothesis that ultra-high-pressure homogenization (from about 50 to 350 MPa) significantly alters properties of oil-in-water emulsions when compared with more traditional pressure treatments.

Material and methods

Ingredients

Whey protein concentrate 'PS85' (85% protein), obtained by ultrafiltration of casein serum, was supplied by Eurial Poitou Touraine (Herbignac, France). The reported composition of this product is: $N \times 6.38$: 85%; fat: 3%; mineral salts: 4%; water content: 4%; pH 6.5 ± 0.2. The proteins are soluble over the complete pH range, and are good emulsifiers at pH < 7.

Sunflower oil was purchased from Lesieur (Neuilly-sur-Seine, France). Distilled water was filtered through a 0.2-μm filter before use. Solutions of whey proteins (1.5%) were prepared using a ultraturrax rotor–stator system (Ika Larbortechnik, Staufen, Germany) and stored at 4 °C.

Ultra-high-pressure homogenizer

Coarse emulsions containing 20%, by mass, of sunflower oil and 80% of the whey protein concentrate aqueous solution were prepared at 4 °C, using an ultraturrax rotor–stator system and then passed through a homogenizer operating from 20 to 350 MPa (Stansted Fluid Power Ltd, Essex, UK). Emulsions were homogenized at different pressures in the range of 50–350 MPa. The homogenizing chamber was cooled with a cooling jacket containing cold water at 5 °C, in order to slow down the rise of temperature. Each emulsion was carefully collected and stored at 4 °C before analysing. This procedure was used to prevent any change in the size distribution of the fat globules because of churning. The experiments were duplicated.

The reproducibility of the high-pressure homogenization was tested by repeating an experimental point (20% oil, 150 MPa) four times.

Light scattering measurements

The size distributions of the oil droplets were determined by the laser light scattering method. The diffractometer model used was the Mastersizer S (Malvern Instruments, Malvern, UK) equipped with a 300 reverse Fourier lens and a He–Ne laser ($\lambda = 633$ nm). The emulsion was measured 5 min after ultra-high-pressure homogenization to cancel any creaming effect, and diluted to about 1/1000 with distilled water in the diffractometer cell, whilst stirring. Size distribution was presented as volume percentage vs. droplet diameter. The volume size distribution was calculated from the intensity of the light diffracted at each angle using Mie theory. The analysis requires a parameter known as the presentation value, a combination of the ratio of the relative refractive indices of the dispersed phase and water and the absorbance of the dispersed phase. The 3NAD presentation was used: oil (1.4564, 0.0000) in water (1.33), for which the absorbance value was selected after consultation with Malvern Instruments and verified using a carefully diluted emulsion of known concentration. The full size distribution was obtained using a polydisperse analysis, which allowed the calculation of the mean droplet diameter d_{32} (Sauter mean diameter) and a dispersion index called 'span', defined as

$$\text{span} = \frac{d[90] - d[10]}{d[50]}$$

where $d[x]$ is the average droplet size in a volume in which $[x]$% of the total sample weight remains constant. Measurements were repeated three times for each sample.

Rheology measurements

Dynamic shear stress measurements were done at 20 °C with an AR 1000 Rheometer (TA Instruments, Waters Corporation, USA), equipped with a coaxial system (medium concentric cylinder with a conical end; R1 = 13.83 mm, R2 = 15.0 mm). Flow curves (shear stress vs. shear rate) were determined at increasing shear rates: 0–1200 s^{-1} in 2 min (up and down flow curves).

Protein surface concentration

Emulsion samples were centrifuged at 13 000 *g* for 30 min to separate the droplets from the aqueous serum phase. The supernatant (the cream) was carefully removed from the aqueous phase using a syringe. The cream layer was resuspended in ultrapure water to wash away any protein trapped between droplets, and the resulting emulsion was centrifuged again at 13 000 *g* for 30 min. The protein concentration of the two serums was determined by the Sigma Pierce spectrophotometric method using bicinchoninic acid reagent (BCA, Sigma procedure TPRO-562). This spectrophotometric method is based on the reduction of Cu^{2+} to Cu^{+} by the proteins (Biuret reaction). Cu^{+} reacts with the BCA and an intense purple complex is formed. This complex produces an absorbance maximum at 562 nm, which is directly proportional to the protein concentration (Smith *et al.*, 1985). A calibration curve was generated using bovine serum albumin (BSA) standard solution (Sigma, St Louis, USA), with a determination coefficient $r^2 = 0.99$ (15 different concentrations used). The quantity of whey proteins absorbed was expressed in equivalent BSA and was calculated from the difference in the serum concentration prior to and after emulsification. The surface concentration of the absorbed protein was calculated from the known surface area per unit volume of emulsion and the difference in the amount of protein measured in the serum phase and the amount used to make the original emulsion, making allowance for any dilution.

Electrophoresis (SDS-PAGE)

The protein fractions were determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), following the procedure described by Arrese *et al.* (1991). Gel slabs were fixed and stained simultaneously in aqueous solution containing 40% v/v ethanol, 7% v/v acetic acid and 0.025% coomassie brilliant blue R250. Proteins were denaturated with a treatment buffer containing 0.125 M Tris, 4% SDS v/v Glycerol, 0.2 M dithiotreitol, 0.02% bromophenol blue. About 50 μg of protein was applied to each gel slot. Molecular weights of the protein bands were estimated by means of the SDS-70L kit (Sigma Chemical Co., St Louis, USA).

Microdifferential scanning calorimetry

Microdifferential scanning calorimetry (μDSC) was used to assess the degree of denaturation of the whey protein by the pressure of homogenization. μDSC thermographs of aqueous solutions of whey proteins before and after a single run in the homogenizer were prepared by using a Setaram 3 calorimeter (Calluire, France). Samples (700 mg) of 2% dispersions of whey proteins (pH 6.7) in distilled water were hermetically sealed in hastelloy C276 pans (volume = 1 cm^3). A closed pan filled with distilled water was used as the reference. The heating rate was fixed at 0.5 °C min^{-1} from 20 to 110 °C. Triplicate samples were analysed by μDSC.

Results and discussion

Whey protein composition

Electrophoresis measurements indicated that the whey proteins were mainly composed of β-lactoglobulin (18 600 Da), α-lactalbumin (14 200 Da), and serum albumin (66 000 Da) (Fig. 1). Examination of the μDSC curves showed three endothermic phenomena (Fig. 2): the first around 40–45 °C, the second between 60 and 65 °C and the third between 70 and 75 °C. These endothermic phenomena in whey proteins are explained by the behaviour of β-lactoglobulin and α-lactalbumin, which are the predominant proteins (Paulsson *et al.*, 1985). The dimer form of β-lactoglobulin exits at 5.2 < pH < 7.5 as in our case (pH 6.7). Thus, according to McKenzie (1971) the first endothermic peak could be a dissociation of the dimers of β-lactoglobulin. The

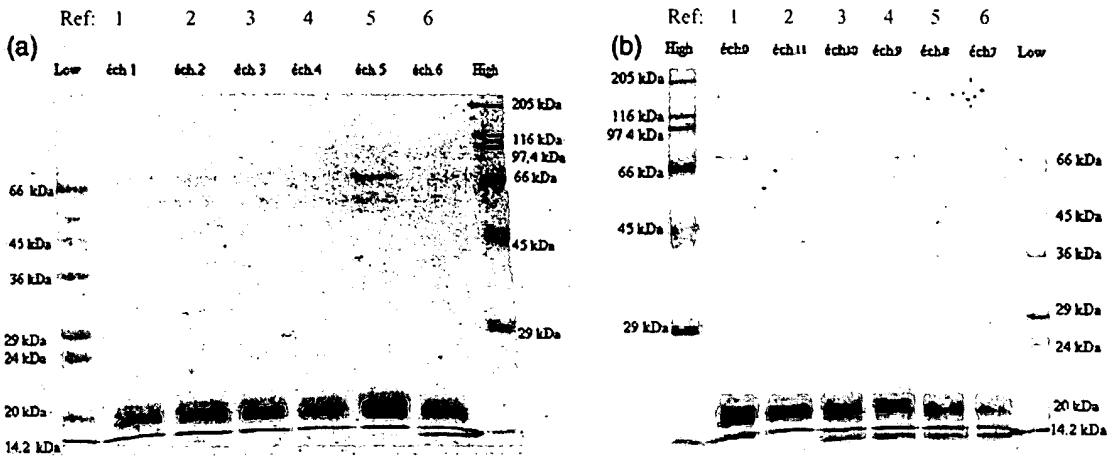

Figure 1 (a) SDS-PAGE of proteins before and after a single run in the homogenizer. Ref: α-lactalbumin (14.2 kDa), tripsin (20 kDa), egg albumin (45 kDa), serum albumin (66 kDa). Column 1: before homogenization. Columns 2–6: after a single run in the homogenizer at 2: 30 MPa; 3: 60 MPa; 4: 90 MPa; 5: 120 MPa; 6: 150 MPa. (b) SDS-PAGE of protein after one run in the homogenizer. Ref: see (a). Column 1: 180 MPa; 2: 210 MPa; 3: 240 MPa; 4: 270 MPa; 5: 300 MPa; 6: 350 MPa.

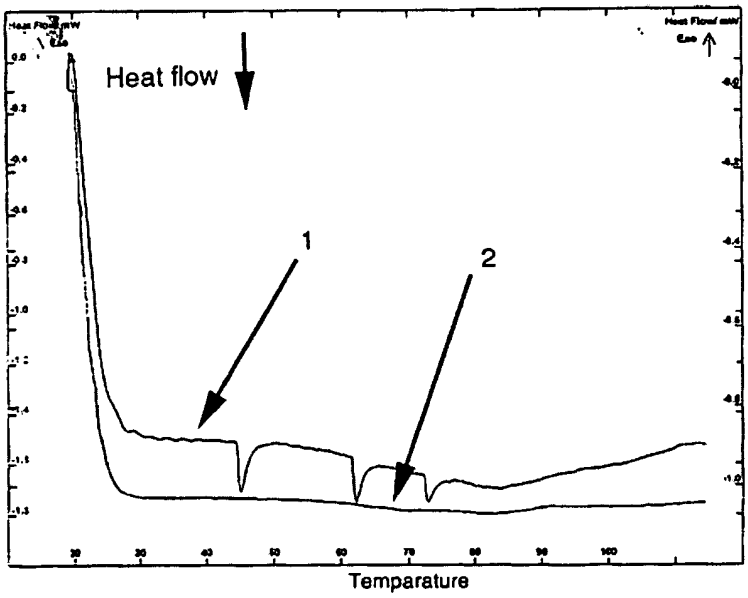

Figure 2 Micro-DSC graph of whey protein aqueous solutions (1.5%) before (1) and after (2) treatment at 300 MPa in the high-pressure homogenizer.

second endothermic peak at 60–65 °C corresponds at pH around 7 to the denaturation of the α-lactalbumin, and the last endothermic peak around 70–75 °C corresponds to the denaturation of the β-lactoglobulin.

Reproducibility of the experiments

The reproducibility of the high-pressure homogenization after testing with an experimental point (20% oil, 150 MPa) and repeated four times on four different days is shown in Table 1. According to the s.d. obtained, the reproducibility was satisfactory.

Evolution of temperature during the experiments

Despite the use of the cooling jacket, the temperature of the emulsion at the exit of the valve increased linearly with the pressure in the valve

Table 1 Reproducibility of the point 150 MPa, 20% oil

Temperature at the exit of the homogenizer	Sauter diameter d_{32} (μm)	Size dispersion coefficient	Viscosity (Pa s)	Fraction of adsorbed proteins (mg m^{-2})
40 °C (1)	0.68 (0.02)	3.75 (0.6)	0.009 (0.001)	0.77 (0.1)

Values within parentheses are standard deviations.

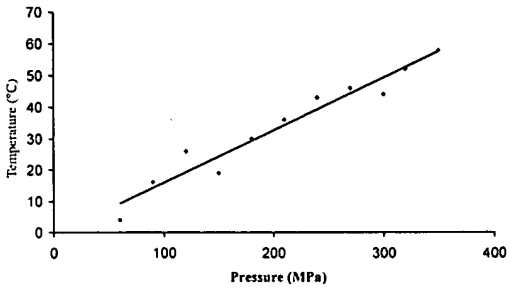

Figure 3 Effect of pressure on the temperature at the exit of the homogenizer.

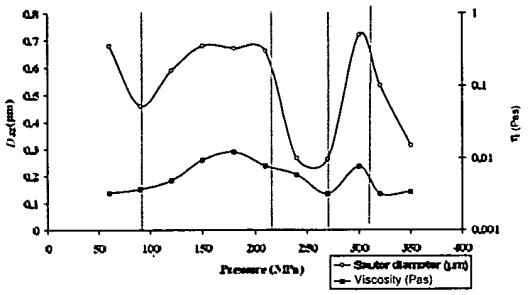

Figure 4 Effect of the pressure of homogenization on the Sauter diameter d_{32} and the viscosity η of the emulsions.

(Fig. 3). Above 300 MPa, the temperature of the emulsion at the exit is close to the temperatures of the peaks observed by μDSC.

Effect of high pressure on emulsion properties

The Sauter diameter before homogenization was around 30 μm. Homogenization reduced the Sauter diameter appreciably, the reduction increasing with treatment pressure from 50 to 90 MPa (Fig. 4). This result agrees with the study of Robin *et al.* (1992), who observed a decrease in the droplet average size between 7.8 and 76.3 MPa. Above 90 MPa, d_{32} increased with pressure (Fig. 4) and then stabilized approaching 200 MPa. Robin *et al.* (1992) observed a similar plateau of the droplet size diameter, but between 60 and 76.3 MPa. This phenomenon can be referred to as 'overprocessing,' as stated by Tornberg (1980): the average droplet size is stable over a certain range of pressure, and increases at higher pressures. The density of energy (up to 10^{12} W m^{-3}) is estimated by knowing the flow rate and the pressure drop inside the valve, and is partially dissipated as heat (Fig. 3), which can lead to an increase of the Sauter diameter: the Brownian motion increases and so also the probability of collision and coalescence.

Above 200 MPa, d_{32} decreased and then increased again at around 250 MPa. However, there was a final decrease of d_{32} above about 300 MPa. This final decrease could be explained by the increased probability of rupture. At this level of pressure, the shear rate inside the valve is enormous, the probability of rupture again becomes higher than the probability of coalescence and d_{32} decreases.

The effects of high-pressure homogenization on the sizes dispersion coefficient are interesting: above 90 MPa, as the pressure increased, the dispersion coefficient strongly decreased (sevenfold) (Fig. 5). Also, the droplet size distribution was bimodal for the lower pressures, but at higher

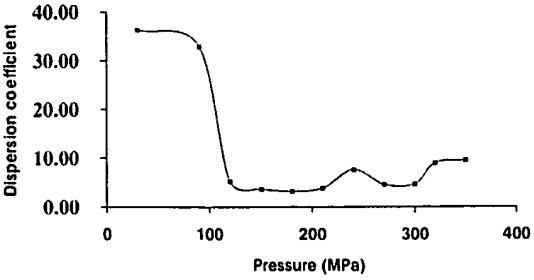

Figure 5 Effect of pressure of homogenization on the size dispersion coefficient of the droplet.

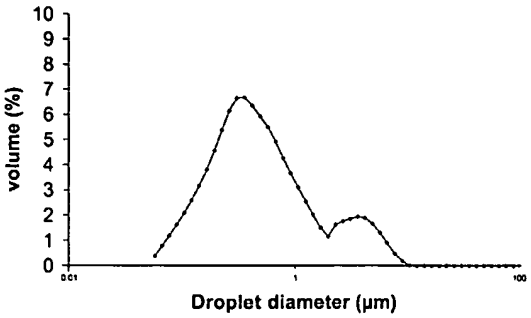

Figure 6 Size distribution of the oil droplet of the emulsion after a single run in the homogenizer at 300 MPa.

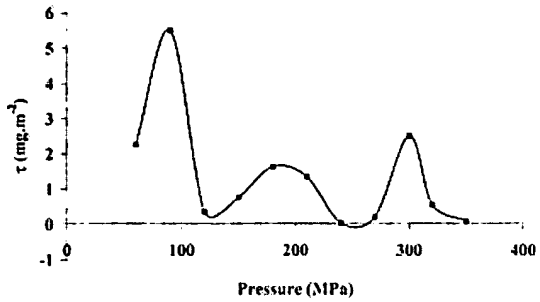

Figure 7 Effect of pressure of homogenization on the fraction of absorbed proteins.

pressures the second peak decreased (Fig. 6). Thus, the coalescence rate of the droplets is reduced because a large dispersion favours rapid coalescence. Above 90 MPa, the main effect of high-pressure homogenization on the size properties is the decrease in the dispersion coefficient of size.

Examination of the rheological properties of the emulsions showed a Newtonian behaviour in all cases, agreeing with previous studies (Desrumaux & Della Valle, 1999). However, the viscosity changed with the pressure in the valve (Fig. 4).

It is interesting to see the similarity of behaviour between the curves for the viscosity η and the Sauter diameter d_{32} (Fig. 4). Both show a complicated behaviour, with four zones, although differences exist in the limits of the zones. In the first zone, up to 90–100 MPa, the Sauter diameter decreased and viscosity increased slowly (Fig. 4). At the same time, the amount of adsorbed protein increased (Fig. 7), as expected, since, as the average droplet size decreases, the specific area increases, which leads to an increase of the fraction of adsorbed proteins. Above 100 and up to ≈210 MPa, d_{32} increased and reached a plateau (Fig. 4), which can be attributed to the 'overprocessing' phenomenon. Simultaneously, the fraction of adsorbed proteins decreased strongly and then increased (Fig. 7). Viscosity followed the same behaviour as d_{32} (Fig. 4). Above 200–210 MPa the behaviour of the structural and textural properties was complicated and, is probably explained, by the effect of the high pressure on the protein conformation. Indeed, studies on isostatic high-pressure treatment of proteins have shown that pressure has a huge effect on food protein functionality (Messens *et al.*, 1997). High-pressure effects on proteins are primarily related to the rupture of noncovalent interactions within protein molecules and to the subsequent re-formation of intra- and intermolecular bonds within or between protein molecules (Smith *et al.*, 2000). As extensively described by Messens *et al.* (1997), the whey proteins, particularly the β-lactoglobulin, are far more sensitive to isostatic pressure than other proteins such as BSA. In our case the treatment at high pressure was very short, estimated at 10^{-4} s from the flow rate and the size of the gap (≈1 μm), but was associated with a significant rise in temperature (Fig. 3). The complicated effects of pressure on emulsions properties could be explained by a change of conformation of the emulsifying whey proteins during the emulsification process. Only a small part of the whey protein is likely to be absorbed at the water/oil interface in the coarse emulsion, because the kinetics of absorption requires a much longer time than that for emulsification. Thus, a large part of the whey protein was in the aqueous phase during the homogenization and the absorption of the whey protein at the interface occurred after homogenization. In order to understand the possible change of the whey protein, we tested aqueous whey protein solutions (1.5%) before and after a single run in the homogenizer at 300 MPa (Fig. 2). Comparing the μDSC graph with the μDSC graph obtained before treatment in the high-pressure homogenizer (Fig. 2), it can be seen that the endothermic peaks disappeared, indicating that the proteins had probably denatured. This phenomenon is because of the combined

effects of the high shear rate inside the gap (velocity at 300 MPa estimated at 500 m s^{-1}) and the rise of temperature observed. At such a high pressure, it was impossible to slow down the rise of temperature inside the valve, the temperature at the exit being directly proportional to the pressure of homogenization. Electrophoresis measurements of extracted proteins after treatment in the high-pressure homogenizer did not show any significant change in the molecular weights (Fig. 1b): the proteins were not broken down into smaller entities during the homogenization, the time of treatment (estimated at 10^{-4} s) probably being too short.

Over 200–210 MPa, the proteins were denatured and had probably partially lost their emulsifying activity (Fig. 4). The effect of the high-pressure homogenization could be protein aggregation; however, the electrophoresis experiments in the presence of SDS prevents observation of protein aggregation.

Conclusions

The effects of high-pressure homogenization on oil in water emulsions are complicated. From 20 to 100 MPa, the Sauter diameter decreased, confirming the results of Davies (1985) and Robin *et al.* (1992). Over 100 MPa, d_{32}, viscosity and the fraction of adsorbed protein displayed up to four zones of behaviour: from 100 to 210 MPa, the Sauter diameter and viscosity increased up to a maximum. This behaviour could be because of the 'overprocessing' phenomenon. Over 210 MPa, μDSC graphs on whey proteins before and after a single run at 300 MPa in the homogenizer confirmed changes in protein conformation, probably because of the combined effects of high-pressure treatment and the rise in temperature observed. This change in the conformation of proteins probably modifies the emulsifying properties of the whey proteins. There is a strong correlation between the formulation of emulsion and the range of pressure used in homogenization. For sunflower oil in water emulsion (20% oil) stabilized with whey proteins, the optimum pressure of homogenization according to the light scattering measurements is ≈100 MPa. At this pressure the d_{32} and the size dispersion coefficient reach a minimum.

Acknowledgments

The authors wish to thank Pierre Cantoni for his technical assistance.

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